FINAL REPORT

PROJECT NO. A-675

BIOLOGICAL EXAMINATION OF FOOD ADDITIVES

By Allen B. Eschenbrenner

Prepared for
Dunn Laboratories
Atlanta, Georgia

June 1963

Engineering Experiment Station
GEORGIA INSTITUTE OF TECHNOLOGY
Atlanta, Georgia

ENGINEERING EXPERIMENT STATION
of the Georgia Institute of Technology
Atlanta, Georgia

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SPECIFIC AIM

To compare the growth and development of weanling rats for a ninety day period on a standard diet and on a standard diet to which had been added two levels each of water soluble (OH-1253) and oil soluble (OH-1254) additives, supplied by Dunn Laboratories.

METHOD OF PROCEDURE

Weanling rats of the Wister strain were obtained from a commercial breeder. There were five dietary groups of 20 males and 20 females each, as follows:

1. Standard diet (converted pellets).
2. Standard diet with 0.3% water soluble additive.
3. Standard diet with 3.0% water soluble additive.
4. Standard diet with 0.025% oil soluble additive.
5. Standard diet with 0.25% oil soluble additive.

The following analysis was given by the manufacturer of the standard diet pellets used:

Crude protein not less than 17.0%

Crude fat not less than 11.0%

Crude fiber not more than 2.0%

Ash no more than 5.5%

INGREDIENTS: Dried skimmed milk, ground wheat, brewers' dried yeast, corn oil, animal fat (preserved with butylated hydroxyanisole), vitamin A oil, D activated plant sterol, 1.4% salt, 0.13% ferric ammonium citrate.

The standard diet pellets were converted into fine granules with the use of a smooth roll crusher and served as the control diet. The additives were incorporated into such granules by Dunn Laboratories. The rats were furnished food and water ad lib. They were housed in galvanized wire cages, five rats per cage. Four cages of a given sex and dietary group were shelved in a vertical fashion to equalize any difference in room temperature gradient between cages on top and bottom shelves of the cage rack. Room temperature was maintained at 76°F. The rats were ear numbered for positive identification. Sterilized, dehydrated, ground corn cobs were used for bedding.

The rats were observed daily for behavior. Individual body weights were recorded weekly. Hemograms were made at six and thirteen weeks (middle and termination of 90-day period) on five males and five females from the control and the two high level diets. This included hemoglobin (Wintrobe modification of Sahli), microhematocrit, total erythrocyte count, and total and differential leucocyte counts.

At the termination of 90 days all rats were sacrificed. The rats on which the hemograms had been made were autopsied and the following organs were weighed: heart, liver, spleen, kidneys, adrenals and male gonads. Of these rats, sections of the following organs were prepared for histopathologic examination: heart, lungs, stomach, small intestine, large intestine, liver, spleen, kidney, adrenal, gonad, and skeletal muscle. The tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6μ and stained with Azure-II-eosin.

RESULTS

Behavior: During the period of daily feeding and watering, there was considerable activity of the rats and this was seen to be the same in all dietary groups. During the early part of the experiment the rats were quite docile during the weekly weighings and during the process of cage-cleaning. As the rats grew older and larger they were a little more difficult to handle, especially the males. Again, no difference was seen, in this respect, between rats in different dietary groups.

Interim Deaths: There were nine spontaneous deaths during the ninety day period. One rat was killed on the 73rd day because of a large abscess of the lateral abdominal wall. This began as a superficial skin infection in the region of the navel, and over a period of three weeks the mass appeared and grew progressively larger. The rat was anesthetized, the mass was incised, and was found to contain much caseous material. The animal was sacrificed because it was not considered a satisfactory subject for a dietary experiment. The data for the ten deaths are tabulated in Table I.

Terminal Data:

Interim and Terminal Data: Weekly body weights*, hemograms at 6 and 13 weeks (middle and termination of 90-day period), and terminal organ weights* are shown in Tables II, III, IV, V, VI, VII, VIII and XI.

HISTOPATHOLOGY

Except for the abnormalities found in the four rats listed in Table X, no significant differences were seen in tissues from rats on five different diets.

*grams

DISCUSSION

When the rats were received from a commercial breeder they were picked up at random when housed five to a cage. The diets furnished by Dunn Laboratories were coded by number. The project director and his assistants did not know of the identity of the diets until the sixth week when this had to be revealed in order to make the hematological observations. It was thus purely chance that male rats on Diet 5 had higher initial and final average body weights.

CONCLUSION

No significant difference was found in the growth and development of weanling rats for a ninety day period on a standard diet and on a standard diet to which had been added two levels of water soluble and oil soluble additives supplied by Dunn Laboratories.

TABLE I

Interim Deaths Before Termination of Experiment

Day	Animal No.	Diet No.	Cause of Death
7	♂ 79	4	Pneumonia
8	♂ 43	3	Lung Abscesses
7	♀ 34	2	*
11	♂ 84	5	*
20	♂ 2	1	Lung Abscesses
21	♀ 37	2	*
23	♀ 19	1	Kidney Abscesses
34	♂ 52	3	Pneumonia
70	♂ 63	4	*
73	♀ 23	2	Killed, Abscess, Abdominal Wall

*Too autolyzed or canabalized to determine cause of death.

TABLE II

Weekly Average Body Weights of 20 Male Rats/Diet

Week No.	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
0	86.9	85.6	87.4	87.0	90.6
1	126.9	127.6	126.7	127.8	132.2
2	166.7	171.2	163.9	163.0	167.4
3	203.6	214.6	202.0	198.6	203.0
4	233.6	243.2	237.9	237.6	238.1
5	272.7	284.5	275.8	270.9	275.5
6	295.6	317.2	302.1	301.5	300.8
7	325.2	333.8	320.9	312.9	317.6
8	341.9	349.4	333.8	325.5	332.6
9	353.3	354.9	343.3	336.4	353.8
10	373.6	377.4	366.7	355.3	367.9
11	379.6	387.8	379.9	367.7	376.6
12	375.2	392.5	389.4	376.9	384.6
13	380.212	391.5	381.312	371.75	384.437

TABLE III

Weekly Average Body Weights of 20 Female Rats/Diet

Week No.	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
0	93.8	88.1	89.8	91.6	89.0
1	129.7	129.4	127.0	129.1	125.1
2	151.1	153.5	144.7	148.9	144.8
3	170.7	174.6	166.2	168.0	168.0
4	189.9	194.3	182.2	182.4	185.3
5	207.9	213.2	198.1	198.1	200.7
6	217.9	226.3	208.7	208.8	211.9
7	227.0	237.2	218.6	219.8	220.4
8	230.9	236.1	221.0	217.7	219.1
9	236.7	240.9	224.5	223.8	227.9
10	238.2	247.9	231.4	225.7	228.1
11	247.5	250.6	233.4	230.9	231.5
12	244.3	251.4	231.0	229.5	233.5
13	253.562	263.50	242.062	240.25	244.5

TABLE IV

Hemograms of Individual Male Rats After 6 Weeks on Diets

Diet No.	Animal No.	RBC	WBC	Hgb.	Hcrt.	Segs.	Lymphs.	Eosin.
1	6	7.13	14,950	16.5	46.5	45	54	1
	7	8.52	12,900	16.25	49.0	8	92	
	8	7.68	19,100	16.5	54.0	6	94	
	9	7.83	39,150	16.0	49.0	28	71	
	10	10.55	13,800	16.5	60.0	17	83	
Average		8.34	19,980	16.35	51.7	20.8	78.8	0.2
3	51	8.40	21,750	16.5	52.0	11	89	
	57	6.90	20,900	16.0	48.0	21	79	
	58	9.74	27,650	16.5	53.0	6	94	
	59	8.22	31,250	16.0	48.0	20	80	
	60	9.04	52,750	16.5	51.0	4	96	
Average		8.46	30,860	16.30	50.4	12.4	87.6	
5	96	8.48	11,100	16.5	46.5	9	92	
	97	5.92	21,950	16.25	47.0	10	90	
	98	7.57	29,300	16.5	49.5	13	87	
	99	8.21	23,300	17.0	49.0	6	94	
	100	8.18	19,100	16.5	48.5	17	83	
Average		7.67	20,950	16.55	48.1	9.5	90.7	

TABLE V

Hemograms of Individual Female Rats After 6 Weeks on Diets

Diet No.	Animal No.	RBC	WBC	Hgb.	Hcrt.	Segs.	Lymphs.	Eosin.
1	6	8.49	11,800	16.0	49.5	14	86	
	7	9.37	34,050	16.5	49.0	7	93	
	8	9.08	12,450	16.5	49.0	26	74	
	9	8.39	20,050	16.5	49.0	12	88	
	10	7.77	14,650	16.0	52.0	8	91	1
Average		8.62	18,600	16.3	49.7	13.4	86.4	0.2
3	41	8.52	19,100	16.5	50.0	15	85	
	42	8.20	20,750	16.0	48.5	18	82	
	51	9.25	28,650	16.0	51.0	10	90	
	53	8.15	25,150	16.0	50.5	7	93	
	54	10.51	16,050	16.5	55.0	20	80	
Average		8.97	21,940	16.2	51.0	14.0	86.0	
5	91	8.04	16,900	16.5	50.0	18	82	
	92	9.34	18,500	16.0	48.5	19	81	
	93	9.32	19,500	16.0	48.0	17	83	
	94	8.62	11,000	16.0	51.0	21	79	
	95	8.36	14,900	16.0	44.0	64	35	1
Average		8.73	16,160	16.1	48.3	27.8	72.0	0.2

TABLE VI

Hemograms of Individual Male Rats After 13 Weeks on Diets

Diet No.	Animal No.	RBC	WBC	Hgb.	Hcrt.	Segs.	Lymphs.	Eosin.
1	6	9.27	15,050	16.0	50.0	18	81	1
	7	6.87	21,000	15.5	53.0	21	79	
	8	6.87	19,450	15.5	49.0	23	75	2
	9	10.88	44,050	16.0	54.0	22	78	
	10	9.76	26,750	14.5	46.5	17	83	
Average		8.73	25,260	15.5	50.5	20.2	79.2	0.6
2	51	10.62	27,550	16.5	53.5	13	87	
	57	9.82	56,850	15.0	49.0	6	94	
	58	10.72	18,000	15.5	56.0	12	88	
	59	9.96	40,200	15.5	50.0	27	73	
	60	7.27	30,600	15.5	52.5	9	91	
Average		9.68	34,640	15.6	52.2	13.4	86.6	
3	96	10.34	41,500	15.0	57.5	16	84	
	97	11.49	19,350	14.5	53.0	26	74	
	98	9.05	36,800	14.5	45.0	6	94	
	99	8.25	26,600	15.0	51.0	17	83	
	100	8.10	30,400	14.5	45.0	10	87	3
Average		9.45	30,930	14.7	49.1	15.0	84.4	0.6

TABLE VII

Hemograms of Individual Female Rats After 13 Weeks on Diets

Diet No.	Animal No.	RBC	WBC	Hgb.	Hcrt.	Segs.	Lymphs.	Eosin.	Mono.
1	6	5.96	13,900	13.0	41.0	12	88		
	7	6.28	27,500	13.0	37.5	10	89		1
	8	4.52	13,350	13.0	40.0	35	65		
	9	5.30	27,050	12.0	37.0	26	69		5
	10	7.12	24,200	15.0	47.0	29	68	1	2
Average		5.84	21,200	13.2	40.5	22.4	75.8	0.2	1.6
2	41	9.00	10,050	14.0	43.0	16	81	1	2
	51	7.40	19,200	16.5	47.0	20	80		
	53	8.81	20,450	12.0	35.0	18	79		3
	54	7.40	19,600	14.5	49.0	27	73		
	55	11.42	9,250	16.0	47.0	14	83	3	
Average		8.81	15,710	14.6	44.2	19.0	79.2	0.8	1.0
3	91	6.88	16,950	14.0	41.5	45	55		
	92	7.67	11,400	14.0	43.0	20	75	2	3
	93	6.91	12,550	13.5	41.5	7	91	1	1
	94	8.36	15,550	14.0	50.0	39	61		
	95	8.50	10,250	14.0	46.0	33	67		
Average		7.66	13,340	13.9	44.4	28.8	69.8	0.6	0.8

TABLE VIII

Organ Weights of Males After 13 Weeks on Diets
Termination of Experiment

Diet No.	Animal No.	Liver	Spleen	Kidneys	Adrenals	Heart	Testes
1	6	9.7	1.1	2.6	0.062	1.15	3.9
	7	12.7	1.2	3.2	0.045	1.05	4.15
	8	11.1	1.0	2.8	0.047	1.05	3.6
	9	10.05	1.7	2.9	0.043	1.15	3.1
	10	12.7	1.5	3.7	0.053	1.05	3.65
Average		11.25	1.3	3.04	0.049	1.09	3.68
3	51	13.9	1.55	3.5	0.052	1.15	3.8
	57	14.5	1.8	2.9	0.051	1.3	3.5
	58	13.4	1.4	2.6	0.040	1.1	3.55
	59	14.1	1.15	2.6	0.038	1.1	3.5
	60	12.1	1.25	2.9	0.042	1.1	3.85
Average		13.6	1.43	2.9	0.045	1.14	3.65
5	96	14.9	1.65	3.2	0.050	1.25	4.15
	97	12.0	1.20	2.55	0.064	1.05	3.35
	98	16.1	1.35	3.0	0.042	1.15	3.9
	99	13.5	1.1	3.05	0.044	1.2	3.65
	100	14.25	1.4	3.25	0.047	1.2	4.25
Average		14.15	1.34	2.95	0.049	1.17	3.86

TABLE IX

Organ Weights of Females After 13 Weeks on Diets
Termination of Experiment

Diet No.	Animal No.	Liver	Spleen	Kidneys	Adrenals	Heart
1	6	7.9	1.5	1.5	0.061	1.05
	7	8.25	1.5	1.8	0.047	1.1
	8	8.7	1.05	1.95	0.062	0.95
	9	8.65	1.5	1.7	0.054	1.0
	10	7.75	1.15	1.5	0.051	0.8
Average		8.25	1.34	1.69	0.055	0.98
3	51	6.70	0.8	1.4	0.051	0.75
	53	7.70	1.2	1.7	0.060	0.8
	54	6.275	1.0	1.4	0.059	0.75
	55	6.70	0.85	1.8	0.067	0.85
	41	7.15	0.875	1.7	0.056	0.80
Average		6.91	0.94	1.6	0.059	0.79
5	91	8.35	1.225	1.75	0.061	0.80
	92	8.10	0.85	1.525	0.056	0.85
	93	9.3	0.85	1.8	0.057	0.90
	94	8.8	1.10	1.75	0.067	0.90
	95	9.3	0.8	1.6	0.052	1.0
Average		8.77	0.96	1.68	0.059	0.89

TABLE X

Histopathology - 13 Weeks

Diet No.	Animal No.	Pathologic Findings
2	♀ 41	Multiple lung abscesses
3	♀ 91	Multiple lung abscesses
3	♀ 95	Peribronchiolar leucocytic infiltration
2	♂ 59	Multiple lung abscesses